D2

thereby obtaining an influenza antigen comprising the fusion product.

D3

54. (Twice Amended) An influenza antigen for an animal species comprising a fusion product, said fusion product comprising

(i) an immunogenic extracellular part of (a) an M2 membrane protein of an influenza A virus or (b) an NB protein of an influenza B virus of said animal species; and

(ii) a heterologous presenting carrier.

REMARKS

Claims 26-32, 34-41, 46, and 52-57 are pending. During the April 11 telephonic interview, the Examiner indicated that she would withdraw the finality of the instant Office Action and enter the amendments requested in Applicants' November 4, 2002 Response. The Examiner also acknowledged that those amendments would overcome the rejections raised in the Office Action.

Pursuant to the Examiner's suggestion made at the telephonic interview, applicants further amend claims 26, 46, and 54 to clarify that the presenting carrier is "heterologous" to the immunogenic extracellular part, i.e., the presenting carrier is an entity that is not natively adjacent to said part. Support for these amendments appears in the specification at, e.g., p. 7.[2]

[2] These amendments do not change the scope of the amended claims. They merely clarify the meaning of "presenting carrier." The claims recite "fusion product," which already indicates

The only remaining issue in this application is the Heinen article applicants submit in the accompanying Supplemental Information Disclosure Statement.[3] Heinen was published after this application was filed, and hence is not prior art. However, since this article pertains to the efficacy of applicants' claimed vaccines, applicants wish to make of record the following remarks.

Heinen describes vaccinating pigs with fusion proteins comprising the extracellular domain of M2 ("M2e"). Two experiments were conducted. The first experiment involved vaccination with a protein vaccine, M2eHBc, where a human M2e sequence was fused to a human hepatitis B virus core protein. The second experiment involved vaccination with a DNA construct encoding M2eNP, where a pig M2e sequence was fused to a pig influenza nucleoprotein. In both experiments, the pigs were challenged with a pig influenza virus after vaccination. According to Heinen, these two experiments show that "antibodies to M2e, especially in combination with cell-mediated immune responses, exacerbate disease" (Abstract). Applicants have reviewed the data in Heinen in detail and found that the data fail to support this conclusion. Neither experiment shows that anti-M2e immunity exacerbates flu symptoms.

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that the extracellular sequence is fused artificially to an entity that is not a native, immediately downstream sequence.

[3] Heinen et al., Journal of General Virology 83:1851-9, 2002.

First Experiment (M2eHBc Protein Vaccination)

The authors allege that "clinical signs after challenge were more severe in all immunized groups compared with the control group" (p. 1854, left col., last full paragraph). In the first experiment, two experimental groups of pigs were used: one group were vaccinated with M2eHBc alone, and the other with M2eHBc plus adjuvant. The so-called control group consisted of six pigs injected with 200 μg of empty plasmids (p. 1853, left col., penultimate paragraph). This "control" was improper because neither experimental group received plasmid vaccination. Two obvious controls were omitted. There was no negative control where a group of pigs were treated with buffer only or with adjuvant only. Nor was there any positive control where a group of pigs were rendered immune to the challenge infection by prior infection or vaccination with a conventional flu vaccine.

Due to the lack of proper controls, it is impossible to verify if the two experimental groups did develop "more severe" clinical signs. It is also uncertain whether these clinical signs were truly infection-related or due to the adventitious effect of the challenge treatment. Indeed, Heinen observed that the group of pigs injected with empty plasmids, the purported control group, developed the highest fever (p. 1854, right col.).

In fact, a close review of Heinen's data reveals that anti-M2e immunity did not exacerbate "clinical signs" of the experimental pigs. The authors report that there was at least a 100-fold higher titer of M2e antibodies in the adjuvanted group than in the non-adjuvanted

group.[4] However, the "clinical signs" and body temperature of the two groups after challenge infection were not statistically different. See, e.g., Figs. 2(A) and 2(B) of Heinen (filled triangles and filled diamonds). In other words, the M2e antibody titer did not correlate with either the "clinical signs" or body temperature of the challenged animals. Thus, Heinen provides no evidence showing that anti-M2e antibodies exacerbated the disease.

The authors assert that the M2eHBc vaccine did not provide any protection. But given that there was a 26% (i.e., 6 out 23 amino acids) mismatch between the human M2e sequence in the vaccine and the pig M2e sequence in the challenge virus (p. 1852, right col., last paragraph), this is not surprising. As applicants discussed in their April 18, 2002 Response, M2e sequences are highly conserved within the same animal species, not among different animal species. Applicants' vaccines are thus developed for species-specific purposes.[5]

Second Experiment (M2eNP DNA vaccination)

The second experiment described in Heinen involved DNA vaccination using a plasmid coding for an M2eNP fusion protein. The authors report that "[i]n the M2eNP DNA group, clinical signs were extremely severe and one pig died on post-challenge day (PCD) 1 and two more died on PCD2 . . . ." (p. 1854, left col., last full paragraph).

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[4] In the non-adjuvanted group, the M2e antibody titer was about 20 at the time of challenge infection. The corresponding titer in the adjuvanted group was about 2000. Heinen, Fig. 3(A).

[5] In the working examples of applicants' disclosure, the mice were exposed to human influenza virus after vaccination with human M2e vaccines.

Like the first experiment, this experiment suffered from the lack of proper controls. Two groups of pigs were used here. The first group were vaccinated with the M2eNP expression plasmid. The second group were vaccinated with an empty plasmid. Heinen, p. 1853, left col., penultimate paragraph. Two crucial controls were missing: a negative control in which the pigs were vaccinated with a plasmid encoding only NP, and a positive control in which the pigs were vaccinated with a conventional flu vaccine.

In the group of pigs vaccinated with an M2eNP-encoding plasmid, 3 out of 6 pigs died 1 or 2 days post challenge. Without proper controls and any histopathology data, it is impossible to draw any conclusion as to the cause of the deaths. Pigs that suffer from an influenza infection usually do not die from it.[6] In any event, Heinen's data suggest that anti-M2e immunity was not the cause. First, Heinen shows that the M2e antibody titers in these pigs were low (about 70; Fig. 3(A), open circles). There is no reason to implicate these low titers in the deaths of the animals. In fact, the anti-M2e titer in pigs vaccinated with M2e-HBc plus adjuvant was far higher (over 2000), and yet no death occurred among those pigs (first experiment, *supra*). Second, Heinen shows that there was no detectable anti-M2e T cell activity in this group of pigs (p. 1857, left col., lines 1-2). Thus, cell-mediated anti-M2e response could not be held responsible, either.

In contrast, the anti-NP antibody titers in this group of pigs were far higher (about 8000; Heinen, Fig. 3 (B), open circles). So were the anti-NP T cell activity levels (p.

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[6] In humans, influenza infection can be fatal, but death typically does not occur on day 1 or 2 post infection.

1857, left col., lines 1-2). But as no control group with an NP-expressing plasmid was included, it remains unknown whether anti-NP immunity or other adventitious events were responsible for the pathology observed in this particular study.

In conclusion, neither the first nor the second experiment provided any evidence showing that anti-M2e immunity exacerbated influenza-induced disease. The "clinical signs" in the experimental groups correlated neither with body temperature[7] nor with virus excretion. In the absence of proper controls and histopathology data, it remains unclear whether the observed clinical signs of the experimental pigs were indicative of the efficacy of M2e vaccines.

Heinen further contends that pigs are a better model for studying the efficacy of influenza vaccines than mice. Applicants disagree. A number of influenza vaccines have been developed successfully without any published reports of tests in pigs. In these cases, the proof of principles and first optimization were done in mice, followed by clinical trials in humans. These vaccines include:

(1) Flumist™, an influenza vaccine approved by the FDA in December 2002 and being marketed by MedImmune, Inc. (http://www.medimmune.com/pipeline/index.asp);

(2) Influvac®, an influenza vaccine marketed by Solvay Pharmaceuticals (http://www.influvac.com); and

[7] As discussed above, the group of pigs vaccinated with an empty plasmid developed the highest fever.

(3) Inflexal V®, an influenza vaccine marketed by Berna Biotech AG (http://www.bernabiotech.com/products/index.html).

In conclusion, Heinen does not provide any evidence that disputes the efficacy of applicants' claimed vaccines.

Applicants respectfully submit that the application as amended is in condition for allowance. The Examiner is invited to telephone applicants' undersigned representatives to resolve any remaining issues in this case.

Respectfully submitted,

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